

REVIEW

The genetic and epigenetic alterations in human hepatocellular carcinoma: a recent update

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ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most frequent human malignancies worldwide with very poor prognosis. It is generally accepted that the progression of HCC is a long-term process with accumulation of multiple genetic and epigenetic alterations, which further lead to the activation of critical oncogenes or inactivation of tumor suppressor genes. HCC is characterized with multiple cancer hallmarks including their ability to proliferate, anti-apoptosis, invade, metastasis, as well as the emerging features such as stem cell properties and energy metabolic switch. The irreversible alterations at genetic level could be detected as early as in the pre-neoplastic stages and accumulate during cancer progression. Thus, they might account for the cancer initiating steps and further malignant transformation. In addition to genetic alterations, epigenetic alterations can affect the cancer transcriptome more extensively. Alterations in DNA methylation, histone modification, miRNAs, RNA editing, and lncRNAs might result in disrupted gene regulation networks and substantially contribute to HCC progression. In this review, the genetic and epigenetic alterations which significantly contribute to the malignant capabilities of HCC will be updated and summarized in detail. Further characterization of those critical molecular events might better elucidate the pathogenesis of HCC and provide novel therapeutic targets for treatment of this deadly disease.

KEYWORDS hepatocellular carcinoma (HCC), cancer hallmarks, genetic regulation, epigenetic regulation, therapeutic targets, HCC progression

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequent human malignancies worldwide. It is the sixth most prevalent cancer in the world and the third leading cause of cancer-related mortality (Parkin et al., 2005). The prevalence of HCC varies markedly in different regions. The highest incidence of HCC was found in Asia-Pacific area (>20/100,000), while low incidence was found in Northern Europe and Northern America (<5/100,000) (Venook et al., 2010). China is a typical high-risk region which may account for more than 50% of HCC cases in the world (Yuen et al., 2009). The uneven distribution of HCC incidence among different geographic regions suggests that multiple genetic and environmental factors may interplay in the progression of this disease. Almost 70%–90% of HCC patients accompany with liver cirrhosis, which is believed to be the most important risk factor for HCC (Fattovich et al., 1997). Thus, all levels of viral infection, liver cytotoxicity, chronic inflammation which can lead to liver cirrhosis are important risk factors in the development of HCC (El-Serag and Rudolph, 2007).

It is widely accepted that carcinogenesis is a multi-step process with accumulation of genetic alterations in critical genes which regulate cell proliferation, growth, survival, apoptosis, adhesion, and metabolism (Vogelstein and Kinzler, 1993). The stepwise accumulation of genetic alterations in oncogenes and tumor suppressor genes will transform a normal cell and finally leads to carcinogenesis (Farber, 1984). The pathogenesis of HCC is also believed to be a long-term process which begins from the pre-malignant stage to the dysplastic stage and finally proceeds to the malignant stage (Thorgeirsson and Grisham, 2002). Better understanding of the genetic and epigenetic changes and their interactions at all the stages during HCC progression

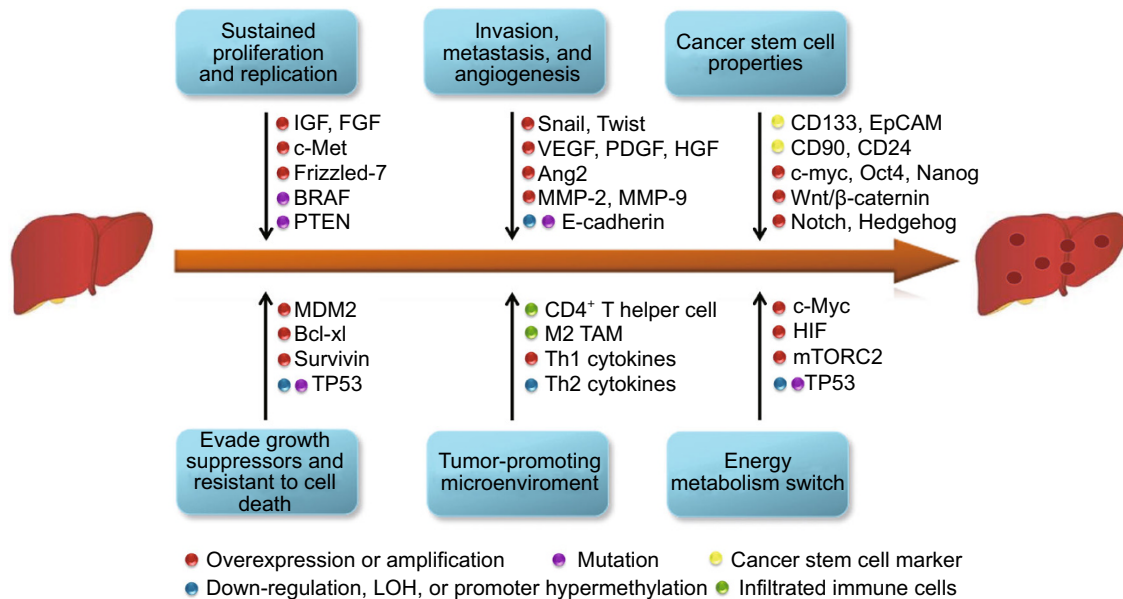


Figure 1. Multiple cancer hallmarks and the underlying molecular alterations in the progression of HCC. The progression of HCC is a multi-step process, which is characterized by several cancer hallmarks including sustained cell proliferation, evading growth suppressors, resistant to cell death, invasion, metastasis, angiogenesis, and deregulated energy metabolism. Multiple cellular and molecular alterations such as amplification or overexpression of oncogenes, hypermethylation or mutation of tumor suppressor genes, activation of cancer stem cells, and infiltration of immune cells, contribute to the malignant transformation of HCC.

will greatly facilitate to elucidate the pathogenesis of HCC. In this review, we summarize the current knowledge of genetic and epigenetic alterations in the progression of HCC.

HALLMARKS OF HUMAN HEPATOCELLULAR CARCINOMA

For decades, scientists are trying to unveil the underlied molecular mechanisms of cancer initiation and progression. However, the diverse characteristics and heterogeneity of cancer usually make confusion. Now, it is widely accepted that cancer evolves progressively from normal cells to malignant stages. During the multistep process, cancer cells acquired several hallmark capabilities which enable them to become tumorigenic and show all kinds of malignant phenotypes (Hanahan and Weinberg, 2000, 2011). Like other solid tumors, HCC is also characterized with those cancer hallmarks such as sustained cell proliferation, evading growth suppressors, resistant to cell death, invasion, metastasis, angiogenesis, and deregulated energy metabolism. The diverse malignant phenotypes of cancer cells usually associate with several genetic or epigenetic alterations of critical oncogenes or tumor suppressor genes. Thus, linking the hallmarks of HCC with genetic or epigenetic alterations will help to identify potential molecular mechanisms and find out novel targets for HCC treatment. Multiple cancer hallmarks and the underlying molecular alterations in the progression of HCC are summarized in Fig. 1.

Sustained proliferation and replication

The growth and proliferation of normal cells are strictly regulated to maintain a homeostasis of cell number and tissue architecture. However, in tumors, cell growth and proliferation are usually deregulated, and sustained cell proliferation is one of the most common traits of cancer cells. Cancer cells have several ways to obtain the ability to proliferate and replicate rapidly. One mechanism is that they can autocrine or paracrine growth factors which will further activate the mitogenic signaling pathways (Lemmon and Schlessinger, 2010). In HCC, cell growth factors such as IGF, FGF were reported to be overexpressed in a way of autocrine or paracrine and further promote cell growth and proliferation (Kim et al., 1998; Yoshiji et al., 2002). Growth factor receptors are usually overexpressed or mutated in tumor cells, which results in persistent activation of downstream mitogenic signals. In HCC, the overexpression of HGF receptor c-Met can activate the downstream Ras/Raf/MEK signaling pathway (Ueki et al., 1997). Overexpression of the Frizzled-7 receptor leads to the activation of Wnt/beta-catenin signaling pathway in HCC (Merle et al., 2004). In addition to direct overexpressing growth factors and receptors, cancer cells can also activate the mitogenic pathways through affecting the upstream and downstream signal transducers or disruption of the negative feed back loop. For example, the oncogenic signal transducer BRAF is frequently mutated and consistently activated in HCC (Colombino et al., 2012); loss

of function mutation of PTEN, which is the negative feedback regulator of PI3K/AKT pathway, was also frequently observed in HCC patients (Yao et al., 1999).

Evading growth suppressors and resistant to cell death

In contrast to the proliferation stimulating signals, cells have also developed a growth inhibition system, which acts as a guardian of cell growth and proliferation. The guardian system is composed of series of growth and proliferation inhibitors, which are usually important tumor suppressor genes. The well-known tumor suppressor TP53 is at the center of the guardian system. Somatic mutation of TP53 is one of the most frequent genetic alterations in human cancer (Olivier et al., 2010). In HCC, TP53 was also found to be frequently mutated, and the common risk factors such as AFB1, HBV, and HCV are reported to cause TP53 mutation (Hussain et al., 2007; Ozturk, 1991). In addition to mutation of TP53 itself, the regulators of TP53 are usually found to be altered in cancer, such as MDM2. MDM2 ubiquitinates TP53 and promotes the proteasome-mediated degradation of TP53 (Kubbutat et al., 1997). Amplification and overexpression of MDM2 was frequently observed in HCC, and this might also account for the deregulated TP53 signaling pathway in HCC (Jablkowski et al., 2005). In addition to cell growth inhibition, cells can undergo apoptosis upon receiving extrinsic or intrinsic signals (Hengartner, 2000). The apoptotic signal is controlled by a group of counteracting pro- and anti-apoptotic proteins. The pro-apoptotic proteins such as Bax and Bak can enhance the permeability of mitochondria membranes and promote the release of cytochrome c, which further activates the caspase cascade. The anti-apoptotic proteins such as Bcl-2, Bcl-xl will counteract the pro-apoptotic proteins (Adams and Cory, 2007). Other regulatory proteins which interfere with the apoptotic signaling cascade, such as survivin, are also important anti-apoptotic components (Adams and Cory, 2007). The overexpression of anti-apoptotic proteins such as Bcl-xl and survivin are frequently observed in HCC patients (Shiraki et al., 2000; Takehara et al., 2001).

Invasion, metastasis, and angiogenesis

Invasion and metastasis is one of the most common hallmarks of cancer, especially those with high grade malignancy. To gain invasive abilities, cancer cells usually undergo morphological changes termed "Epithelial-to-Mesenchymal transition" (EMT). In epithelial tissues, cells are usually attached to each other or to the extracellular matrix (ECM) through adhesion molecules. However, in metastatic tumors, the adhesion molecules such as E-cadherin are usually down-regulated or mutated, and the loosened cell contact enables tumor cells to invade out from the primary niche (Cavallaro and Christofori, 2004). Conversely, adhesion molecules associated with cell migration such as N-cadherin, which are usually expressed in migrating cells,

will be up-regulated in the aggressive cancer cells. The morphology of the cell will also change from the epithelial-like phenotype to fibroblastic mesenchymal-like phenotype (Voulgari and Pintzas, 2009). In HCC, altered expression of E-cadherin was frequently observed and correlated with clinical pathological features (Wei et al., 2002). Loss of heterozygosity (LOH) and CpG island hypermethylation have been proved to be the major mechanisms accounting for E-cadherin inactivation in HCC (Kanai et al., 1997). To date, several important transcriptional factors, such as Snail, Slug, Twist, and Zeb1/2, have been proved to be the key regulator of the EMT process. Overexpression of Snail and Twist has been closely correlated with HCC metastasis through inducing EMT (Lee et al., 2006; Sugimachi et al., 2003). In order to migrate from the original tissue, cancer cells need to degrade the barriers which hinder their movement, such as extracellular matrix. Matrix metalloproteinases (MMP) is a kind of secreted protease family, which can help digest the ECM (Stamenkovic, 2000). MMPs are synthesized in an inactive form, which could be activated after removing the pro-peptide domain (Pei et al., 2000). Overexpression and activation of MMPs are frequently observed in cancer cells, especially those with high metastatic ability (Rundhaug, 2003). Overexpression of MMPs, such as MMP-2 and MMP-9, are frequently observed in HCC patients and has been associated with cancer invasive potential (Arii et al., 1996; Giannelli et al., 2002).

Angiogenesis is another important feature of cancer. When tumor mass grows, the tumor cells need blood vessels to provide enough nutrient and oxygen. Formation of tumor vessels can accelerate the proliferation, growth, and metastasis of cancer cells (Carmeliet and Jain, 2000). In the process of angiogenesis, tumor cells will secrete several critical growth factors such as FGF, VEGF, which will activate the proliferation of endothelial cells or fibroblasts (Yancopoulos et al., 2000). VEGF has been proved to play a critical role in tumor angiogenesis including HCC. Overexpression of VEGF was correlated with HCC angiogenesis and vascular formation (Mise et al., 1996). Monoclonal antibodies targeting VEGF or small molecules inhibiting VEGF receptors have already being used in HCC treatment (Finn and Zhu, 2009). In addition to VEGF, other proangiogenic factors including platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), and angiopoietin-2 (Ang2) are also elevated in the HCC plasma and make a substantial contribution to HCC angiogenesis (Semela and Dufour, 2004; Zhu et al., 2011).

Tumor-promoting microenvironment

The initiation, growth, and metastasis of tumor not only depend on the malignant characteristics of cancer cells themselves, but also the tumor-promoting microenvironment (Joyce, 2005). Tumor grows in a complicated microenvironment, which is composed of stromal fibroblasts, endothelial

cells, and infiltrating immune cells. These cells in the micro-environment can either secrete growth factors to support tumor cell growth and angiogenesis, or produce pro-inflammatory cytokines and chemokines, which favors malignant transformation (Whiteside, 2008). For HCC, the tumor microenvironment might play a critical role in tumor initiation and progression. Etiological studies indicated that HCC mainly developed from liver cirrhosis, which is caused by chronic hepatitis virus infection, fatty liver disease, and alcohol abuse. The common trait during hepatocarcinogenesis is the sustained liver damage and regeneration, which leads to an inflammatory microenvironment in the liver. The inflammatory microenvironment supports the recruitment and activation of hepatic stellate cells and macrophages, which further produce components of the ECM, growth factors, and chemokines for angiogenesis and fibrosis (Hernandez-Gea et al., 2013).

There are several kinds of cells closely associated with HCC tumor microenvironment. Like other solid tumors, the most common cells observed in the tumor microenvironment are immune cells. In response to inflammatory signal, immune cells including T cells, B cells, macrophages, and dendritic cells will infiltrate into the tumor mass, and produce several kinds of cytokines, which either inhibit or promote tumor growth (Hernandez-Gea et al., 2013). The most common tumor-infiltrating lymphocyte is CD4⁺ T helper cells. The cytokines secreted by Th cells could further be divided into two groups including Th1-like cytokines and Th2-like cytokines. A unique signature of increased Th1 cytokines (IL-1, IL-2, TNF α , etc.) but decreased Th2 cytokines (IL-4, IL-8, IL-10, etc.) was frequently observed in HCC tumor microenvironment and associated with poor prognosis of HCC patients (Ye et al., 2003). Tumor associated macrophage (TAM) is another important subset of infiltrated immune cells in the tumor microenvironment. High density of infiltrated TAMs usually associated with poor prognosis of HCC patients (Ding et al., 2009). TAMs can either secrete tumor-promoting growth factor, cytokines, chemokines, etc. to facilitate tumor growth, or suppress the anti-tumor immunity in HCC tissues. The macrophages can also be divided into two subgroups like T helper cells. TAMs resemble the M2 macrophages, which provide the formation of Th2 tumor microenvironment (Bingle et al., 2002).

Cancer stem cell properties

According to the cancer stem cell (CSC) model, cancer originates from a subset of stem like cells that have self-renewal properties. Malignant cancer cells usually have similar properties as embryonic cells characterized with elevated stemness markers and maintained in a dedifferentiated status (Reya et al., 2001). Assessing the differentiation level of tumor is often conducted in the clinic, and the poorly-differentiated tumors are closely associated with patient prognosis. The histologically poorly-differentiated tumors usually show an embryonic-like gene expression

signature (Ben-Porath et al., 2008). In HCC, certain cell populations have been identified as potential cancer stem cells. The “Oval cells” which give rise to hepatoblast cells and primitive bile duct cells during liver development are considered to be origins of liver cancer stem cells. Oval cells express cellular markers of both hepatocytes and bile duct, and have the potential to differentiate into both lineages. Therefore, oval cells are considered to be liver progenitor cells, which might be initiating cells in hepatocarcinogenesis (Mishra et al., 2009). A small group of cells known as “side population” (SP), which are able to pump out nucleus dye via ABCG2-transporters, are also considered to be potential liver cancer stem cells. SP cells have enhanced self-renewal ability *in vitro* and tumorigenic ability *in vivo*. Molecular characterization of SP cells indicated that several oncogenic signaling pathways associated with cancer self-renewal and differentiation, are activated in SP cells (Marquardt et al., 2011). In addition to oval cells and SP cells, several cell surface markers have been identified as cancer stem cell markers, including CD133, EpCam, CD90, CD24, etc. The isolated cancer cells using those markers all shown strong self-renewal properties and tumorigenic ability with only few cells injected in xenograph mouse model (Lee et al., 2011; Ma et al. 2007; Yamashita et al., 2009; Yang et al., 2008).

Several oncogenic signaling pathways have been proved to play important roles in regulating cancer stemness and differentiation. The well-known oncogene c-myc was reported to account for the embryonic stem cell like phenotype of cancer cells (Kim et al., 2010). Inactivation of the myc network can induce the differentiation of HCC cells (Shachaf et al., 2004). Wnt/ β -catenin is another important signaling pathway in regulating cancer stemness and differentiation. The secreted wnt will inhibit the cytoplasmic degradation of β -catenin, which further activates the β -catenin/TCF transcriptional machinery and promotes the transcription of several stemness markers such as Epcam, Ck19, and CD44, etc. (Fodde and Brabletz, 2007; Yamashita et al., 2007). In addition, other critical signaling pathways involved in regulating stem cell self-renewal and differentiation, such as Oct4, Nanog, Sox2, and Notch/Hedgehog have been reported to be important in maintaining the pluripotency of liver cancer progenitor cells and are frequently activated in HCC (Patil et al., 2006; Yuan et al., 2010).

Energy metabolism switch

Altered energy metabolism switch from oxidative phosphorylation to glycolysis, known as Warburg effect, has been widely accepted as an emerging hallmark of cancer. Early in the 1930s, oncologists and scientists have noticed the alteration of energy metabolism in malignant tumors. Glycolysis was preferentially used as the main program for energy metabolism in tumor cells even in the presence of oxygen (Cairns et al., 2011). In normal cells, ATP is mainly generated from the tricarboxylic acid (TCA) cycle, followed

by oxidative phosphorylation in the mitochondria. Oxidative phosphorylation generates 36 molecules of ATP from one molecule of glucose. In contrast, the glycolysis only generates two molecules of ATP from one molecule of glucose (Kroemer and Pouyssegur, 2008). Although glycolysis is not efficient in generating energy, it can provide a large amount of nucleotides, fatty acids, membrane lipids to support the synthesis of macromolecules, which are required for rapid tumor growth. In compensation, cancer cells increased the glucose intake by up-regulating glucose transporters and enhancing the usage of glutamine. The Warburg-like metabolic switch found to be present in many rapidly dividing embryonic tissues further supported the hypothesis that glycolysis could generate diverse intermediates for biosynthetic programs that are important for active cell proliferation (Hsu and Sabatini, 2008).

The reduced dependence of cancer cells on oxidative phosphorylation is not only due to defects in the cellular components of TCA cycle, but also strictly regulated by series of oncogenes and tumor suppressor genes. Oncogenic activation of c-myc, Ras, Akt, and HIF, or inactivation of tumor suppressors such as TP53 can drive metabolism changes in cancer cells (Levine and Puzio-Kuter, 2010). Like other solid tumors, metabolic remodeling from mitochondrial oxidation to aerobic glycolysis is common in human HCC (Beyoglu et al., 2013). c-Myc was reported to induce mouse liver tumors with elevated glucose and glutamine catabolism (Yuneva et al., 2012). Hepatic mTORC2 can activate glycolysis and lipogenesis through phosphorylating AKT (Hagiwara et al., 2012). Multi-kinase inhibitor such as sorafenib, which targets those oncogenic signaling pathways, was reported to be able to reverse the metabolic reprogramming in HCC (Fiume et al., 2011).

GENETIC ALTERATIONS IN HCC

Genetic alteration is one of the most important mechanisms associated with HCC initiation and progression. Genetic changes could be observed as early as in the pre-neoplastic lesions of cirrhotic liver, and it is thought to be the initiating events in hepatocarcinogenesis. The irreversible genetic abnormalities accumulate in hepatocytes, which further cause disrupted gene expression, and finally lead to malignant transformation. Genetic alterations could be divided into several types, including large chromosomal amplification, translocation, deletion, small fraction loss, and single nucleotide variation. The genetic changes at all levels usually result in the activation or loss-of-function of certain important oncogenes or tumor suppressor genes, which govern cell growth and proliferation.

Chromosomal instability

Chromosomal instability is the most common genetic changes in HCC. It could be induced by either error during mitosis

or disruption in DNA replication and repair. The chromosome abnormalities could be observed as the gain and loss of whole chromosome arms, or just amplification and deletion of small chromosomal fragments. According to the comparative genomic hybridization (CGH) data, chromosome 1q and 8q are frequently amplified, while chromosome 1p, 4q, 6q, 9p, 16p, 16q, and 17p are frequently lost in HCC (Guan et al., 2000). The observation of chromosomal alterations in preneoplastic liver tissues indicated that chromosome instability may occur in the early stage of HCC, and accumulates during tumor progression (Kondo et al., 2000). Thus chromosome instability may activate certain cancer driver genes during hepatocarcinogenesis.

Amplification of chromosome 1q is one of the most frequently observed chromosome abnormalities in HCC. The minimal region of 1q21 was found to be amplified in more than 50% of HCC patients. A well characterized oncogene CHD1L is localized in that region (Ma et al., 2008). CHD1L was found to have several oncogenic roles such as inhibiting cell apoptosis, regulating cell mitosis, and promoting cell epithelial-to-mesenchymal (EMT) transition during hepatocarcinogenesis (Chan et al., 2012; Chen et al., 2010; Chen et al., 2009a). In a transgenic mice model, CHD1L could induce spontaneous liver tumors formation (Chen et al., 2009b). In addition, CHD1L could regulate p53 stability, potentially via interacting with SCYL1BP1, which modulates the pirh2-mediated ubiquitin degradation of p53 (Hu et al., 2012). Adenovirus-mediated silencing of CHD1L could inhibit HCC tumorigenesis in xenograft mouse model further suggested CHD1L as a potential therapeutic target in HCC treatment (Chen et al., 2011). In addition to chromosome 1q21, a recent study indicated that a novel potential oncogene Maelstrom (MAEL) at 1q24, could induce EMT and enhance stemness properties of HCC cells (Liu et al., 2013). Chromosome 8q is another highly amplified chromosome arm in HCC, especially at the 8q24 region (Wang et al., 2002). Well-known oncogenes including *c-Myc* and *PTK2* are located at this region, and have been characterized for their oncogenic effects on HCC development (Okamoto et al., 2003; Santoni-Rugiu et al., 1998). In addition to 8q24, the chromosomal region proximal to the centromere is also frequently amplified in HCC (Parada et al., 1998). A serine/threonine kinase SGK3, which shares great similarity with AKT, was found to be frequently amplified and confer AKT-independent oncogenic roles in HCC (Liu et al., 2012).

Chromosome segmental loss is also frequently observed in HCC. The minimal region of 1p35-36 was found to be deleted in more than 50% HCC patients. Several tumor suppressors such as 14-3-3 σ and Rb-interacting zinc finger 1 (RIZ1) were located in this region (Iwata et al., 2000). Loss of the short arm of chromosome 8 has been recurrently observed in HCC. A minimal region of 8p21-22 was found to be frequently deleted in HCC. Deleted in liver cancer 1 (DLC-1), which is a homolog of the rat RhoGAP gene, is located in that region (Yuan et al., 1998). DLC-1 is frequently deleted in HCC tissues due to allele

Table 1. Aberration of chromosome and candidate target genes reported in HCC.

Chromosome	Type of aberration	Frequent aberration region and candidate target genes (Location)	References
1q	Gain	<i>CKS1B</i> (1q21.2), <i>CHD1L</i> (1q21.1), <i>JTB</i> (1q21), <i>MDM4</i> (1q32.1)	Chen et al., 2010; Kim et al., 2008
1p	Loss	<i>p18</i> (1p32), <i>14-3-3σ</i> (1p35), <i>p73</i> (1p36.3), <i>RIZ</i> (1p36.13-p36.23)	Nishimura et al., 2005; Iwata et al., 2000; Fang et al., 2000
3q	Gain	<i>Gankyrin</i> (3q28)	Higashitsuji et al., 2000
3p	LOH, CpG methylation	<i>RASSF1A</i> (3p21.3), <i>CTNNB1</i> (3p21), <i>TGF-1βR11</i> (3q22)	Zhang et al., 2002; Miyoshi et al., 1998
4q	LOH		Zondervan et al., 2000
6p	Gain		Chochi et al., 2009
6q	LOH	<i>M6P/IGF2R</i> (6q26-q27)	Oka et al., 2002
8q	Gain	<i>c-Myc</i> (8q24.21), <i>PTK2</i> (8q24.3), <i>EIF3S3</i> (8q23.3), <i>SGK3</i> (8q13.1)	Santoni-Rugiu et al., 1998; Okamoto et al., 2003; Liu et al., 2012
8p	LOH, CpG methylation	<i>DLC-1</i> (8p21.3-22), <i>LPTS</i> (8p23), <i>CSMD1</i> (8p23.2)	Yuan et al., 1998
9p	LOH, CpG methylation	<i>CDKN2A</i> (9p21), <i>CDKN2B</i> (9q21),	Wang et al., 2000
10q	LOH	<i>PTEN/IMMAC1</i> (10q23.3)	Fujiwara et al., 2000
11q	Gain	<i>cyclinD1</i> (11q13)	Nishida et al., 1994
11p	LOH, CpG methylation	<i>KAI1</i> (11p11.2), <i>IGF-2</i> (11p15), <i>TSLC1</i> (11q23.2)	Tsujiuchi et al., 2007
13q	LOH	<i>Rb1</i> (13q14.2), <i>BRCA2</i> (13q12.3), <i>Tg737</i> (13q12.1), <i>TFDP1</i> (13q34), <i>CUL4A</i> (13q34), <i>CDC1</i> (13q34)	Kuroki et al., 1995; Yasui et al., 2002
16q	LOH, CpG methylation	<i>CDH1</i> (16q22.1)	Wang et al., 2000
16p	CpG methylation	<i>Axin1</i> (16p13.3), <i>SOCS-1</i> (16p13.3)	Li et al., 2013c; Ko et al., 2008
17p	LOH	<i>p53</i> (17p13.1), <i>HIC-1</i> (17p13.3), <i>HCCS1</i> (17p13.3)	Nishida et al., 1993; Kanai et al., 1999; Zhao et al., 2001

loss and promoter hypermethylation (Wong et al., 2003). Restore DLC-1 expression in hepatoma cells could induce cell apoptosis, and inhibit tumor growth (Zhou et al., 2004). Chromosome 16q is another region with frequent deletion in HCC. Cell adhesion molecule E-cadherin (CDH1), which inhibits cell proliferation and metastasis, is located on 16q22 (Kanai et al., 1997). Another tumor suppressor gene Tyrosine aminotransferase (TAT), which might contribute to the pathogenesis of HCC, is also located on 16q22 (Fu et al., 2010). Recently, a significant allele-specific imbalance was identified in the 16q23 region in a cohort of HCC patient due to LOH. The affected gene *Oxidative Stress-Induced Growth Inhibitor 1* (*OSGIN1*) can directly induce cell apoptosis in HCC cells and contributes significantly to the progression of HCC (Liu et al., 2014). The well-known tumor suppressor TP53 is mapped to 17p13.1, which is also a recurrently lost region in HCC. The 17p13 region was characterized with DNA hypermethylation, and loss of 17p13.1 was closely associated with TP53 mutation (Nishida et al., 1993). Summary of chromosome alterations and candidate target genes reported in HCC was listed in Table 1.

Genomic mutations

In addition to large chromosomal alterations, genomic mutation is another important genetic alteration which contributes to tumor initiation and progression. Genomic mutations could be divided into germline mutations and somatic mutations. A germline mutation is usually inherited, and exists in all cell types of a body. Germline mutations are rare, and usually account for cancer risk in certain families. In contrast, somatic mutations usually exist in tumor tissues or preneoplastic tissues, and accumulate during cancer progression. Somatic mutations are more common, and might account for malignant transformation of sporadic tumors. Missense genomic mutations at the open reading frame can either lead to loss-of-function of tumor suppressors or gain-of-function of oncogenes. In addition, mutations at the non-coding region of the genome can also affect cancer risk and progression, for they may change the transcription, translation, or stability of the gene product.

With the development of the next-generation high throughput deep sequencing technology, scientists now are

able to identify somatic mutation patterns in a certain tumor tissue, like HCC. Recently, two groups have sequenced the whole genome of several HCC tumor tissues and their paired non-tumor tissues (Fujimoto et al., 2012; Guichard et al., 2012). Recurrent somatic mutations were enriched in several signaling pathways including wnt/ β -catenin, p53/cell cycle control, chromatin remodeling, PI3K/Ras signaling, and oxidative and endoplasmic reticulum stress. The wnt signaling pathway was found to be the most frequently altered in HCC. Activating mutation in CTNNB1 was found in 32.8% of HCC patients. While the inactivating mutations of AXIN1 and APC was found in 15.2% and 1.6% of HCC patients, respectively. The second most altered pathway in HCC is the p53 signaling pathway. Inactivation mutation of p53 was present in 20.8% of HCC patients and mutation in CDKN2A was identified in 8% of HCC patients. In addition to other traditional signaling pathways, which are frequently mutated in cancer, several recent studies reported that the components of the chromosome remodeling complex are frequently mutated in many cancer types including HCC. The mutation of the SWI/SNF chromatin remodeling complex component ARID1A was detected in more than 20% of HCC patients. These indicated that the chromatin remodeling complex might play important roles in cancer initiation and progression.

Cancer susceptibility genes

It is widely accepted that genetic polymorphisms at cancer susceptibility genes can affect the cancer risk of certain population. Unlike genetic mutations, which directly cause loss-of-function or gain-of-function of gene products, and usually affect important oncogenes or tumor suppressor genes involved in critical signaling pathways, nucleotide changes in cancer susceptibility alleles might not directly cause dramatic functional changes of a protein. Instead, cancer susceptible genetic variations might slightly affect the function of a protein, for example the efficiency of an enzyme, thus confer an increased cancer risk for certain population. A wide range of genes are associated with cancer risk, including carcinogen metabolism genes, anti-tumor immune response genes, and genes associated with cellular response to stress (Antoniou et al., 2010).

Genome-wide association study (GWAS) is emerging as a powerful tool to identify cancer susceptibility alleles in tumorigenesis. GWAS examines common genetic variants in different individuals and identifies variants associated with certain disease. In contrast to mendelian linkage analysis, which aims to identify highly penetrant tumorigenic mutations, GWAS is powerful in identifying less penetrant tumor susceptibility alleles, which are more common and might be important in cancer initiation and progression. Several GWASs have been performed to identify susceptibility alleles associated with HCC. Intronic SNP (rs17401966) in KIF1B on chromosome 1p36.22 has been linked to HBV-

associated HCC (Zhang et al., 2010). Chromosome loci 6p21.32 and 21q21.3 have also been associated with HCC in chronic HBV carriers (Li et al., 2012). A recent study indicated that genetic variations in STAT4 and HLA-DQ genes may confer risk of HBV-related HCC (Jiang et al., 2013). SNP (rs2596542) in the 5' flanking region of MICA on 6p21.33 has been linked to HCV-associated HCC (Kumar et al., 2011).

MicroRNAs (miRNAs)

MicroRNA, a class of non-coding RNAs, has been identified as important regulators of gene expression at post transcriptional levels. Emerging evidences indicated that miRNAs are associated with the development and progression of HCC. In recent years, intensive investigations have been conducted to find out the abnormally expressed miRNAs and their roles in HCC development and progression. Some miRNAs can regulate the proliferation pathways via modulating cyclins or cyclin-dependent kinases, such as miR-122a and miR-221 (Gramantieri et al., 2007). Some miRNAs can help HCC cells to escape from apoptosis by targeting pro-apoptotic protein. For example, Bmf, a proapoptotic protein, is a target of miR-221 (Gramantieri et al., 2009). On the contrary, other miRNAs can promote HCC apoptosis. For example, the anti-apoptotic proteins Bcl-2 and Mcl-1 are two direct targets of miR-29 (Xiong et al., 2010). As two of the most critical hallmarks of HCC, invasion and metastasis are also regulated by miRNAs. On the one hand, the pro-metastatic miRNAs can promote cell migration and spreading in HCC. For example, miR-106b can promote HCC cell migration and invasion by activating epithelial-mesenchymal transition (EMT) process (Yau et al., 2013). On the other hand, several miRNAs such as let-7g, miR-139, and miR-195 can suppress metastasis and progression of HCC (Ji et al., 2010; Wang et al., 2013b). Additionally, some miRNAs have been reported to enhance the ability of self-renewal and tumorigenicity of HCC. MiR-130b can regulate CD133(+) liver cancer stem cells via silencing TP53INP1 (Ma et al., 2010). Inhibition of miR-181 can result in a reduction in EpCAM(+) HCC cell quantity. Exogenous miR-181 expression in HCC cells led to an enrichment of EpCAM(+) HCC cells and promote tumor initiating ability (Ji et al., 2009). Summary of the abnormally expressed miRNAs and their functions are listed in Table 2.

RNA editing

The RNA transcripts are usually faithfully transcribed from the genome without sequential changes after RNA processing. However RNA editing is a molecular process which could result in nucleotide changes at specific sites of the RNA transcripts. Thus, RNA editing could add great diversity to the posttranscriptional regulation of gene expression (Gott and Emeson, 2000). RNA editing can modify the transcribed RNA sequences via nucleotide insertion, deletion, and substitution. The most common type of RNA editing in human is

Table 2. MiRNAs aberrantly expressed and validated target genes in hepatocellular carcinoma.

miRNA	Expression	Gene targets	Function	References
miR-17-5p	Up	p38, MAPK pathway, E2F-1, c-MYC	Promote tumor growth and metastasis.	Yang et al., 2010; El Tayebi et al., 2013
miR-18a	Up	ER α	Promote proliferation.	Liu et al., 2009
miR-18b	Up	TNRC6B	Promote cell proliferation and loss of cell adhesion.	Murakami et al., 2013
miR-21	Up	PTEN, RECK, PDCD4	Inhibit apoptosis, promote cell migration and invasion.	Meng et al., 2007; Zhou et al., 2013
miR-106b	Up	E2F1, RhoGTPases, RhoA, RhoC	Promote cell migration and stress fiber formation.	Yau et al., 2013
miR-130b	Up	TP53INP1	Promote CD133(+) liver cancer stem cell growth and self-renewal.	Ma et al., 2010
miR-143	Up	FNDC3B	Promote tumor metastasis.	Zhang et al., 2009
miR-151	Up	RhoGDI α , FAK,	Stimulate tumor invasion and metastasis.	Ding et al., 2010; Luedde, 2010
miR-181b	Up	TIMP3	Promote tumor metastasis.	Wang et al., 2010a
miR-181	Up	CDX2, GATA6,NLK	Promote EpCAM(+) liver cancer stem cell growth and self-renewal.	Ji et al., 2009
miR-185	Up	KCNN3	Association with HCC venous metastasis.	Budhu et al., 2008
miR-210	Up	VMP1	Promote hypoxia-induced HCC cell metastasis.	Ying et al., 2011
miR-221/222	Up	CDKN1B/p27,CDKN1C/p57, DDIT4, PTEN, Bmf, TIMP3, PPP2R2A	Inhibit apoptosis, promote tumor growth and metastasis.	Fornari et al., 2008; Gramantieri et al., 2009
miR-224	Up	API-5, CDC42, CDH1, PAK2, BCL-2, MAPK1, PPP2R1B.	Promote cell proliferation, migration, invasion, and inhibit cell apoptosis.	Wang et al., 2008; Zhang et al., 2013
miR-1	Down	FoxP1, MET, HDAC4.	Inhibition of cell growth and reduced replication potential.	Datta et al., 2008
let-7	Down	c-Myc, p16, Bcl-xl, COLIA2.	Inhibition of cell growth and proliferation.	Wang et al., 2010b; Ji et al., 2010
miR-26a	Down	Cyclin D2, Cyclin E2,Cyclin E1, CDK6, IL-6	Inhibit tumor growth, metastasis, and invasion.	Yang et al., 2013
miR-29	Down	MEG3, Bcl-2, Mcl-1	Promotion of apoptosis and inhibition of tumor growth	Xiong et al., 2010
miR-34a	Down	c-Met	Inhibition of cell growth, migration, and invasion.	Li et al., 2009
miR-122	Down	CyclinG1, ADAM10, SRF, IGF1R, PTTG1, PBF,CUTL1, NDRG3, MDR-1	Inhibit viral replication and cell proliferation.	Song et al., 2012; Li et al., 2013a; Xu et al., 2010; Gramantieri et al., 2007
miR-124	Down	ROCK2, EZH2, PIK3CA	Inhibit tumor growth, invasion, and metastatic potential of HCC.	Zheng et al., 2012; Lang and Ling, 2012
miR-126	Down	ROCK2, c-Fos	Inhibit cell invasion and migration.	Wong et al., 2011

Table 2 continued

miRNA	Expression	Gene targets	Function	References
miR-145	Down	OCT4, IRS1, IRS2, IGF signaling, HDAC2.	Inhibit cell proliferation, migration, and invasion.	Wang et al., 2013a; Law et al., 2012; Noh et al., 2013
miR-148a	Down	HPIIP, AKT/ERK/FOXO4/ATF5 pathway	Inhibit tumorigenesis.	Xu et al., 2013
miR-195	Down	cyclin D1, CDK6, E2F3, LATS2, VEGF, VAV2, CDC42, IKK α and TAB3, TNF- α /NF- κ B pathway	Inhibit G1/S transition, angiogenesis, and metastasis, promote apoptosis.	Xu et al., 2009; Wang et al., 2013b; Ding et al., 2013
miR-199a-3p	Down	mTOR, c-Met, CD44	Inhibit cell growth and metastasis	Fornari et al., 2010; Henry et al., 2010
miR-214	Down	XBP-1, HDGF, EZH2, CTNNB1, β -catenin signaling pathway	Inhibit cell proliferation, promote cell apoptosis, and suppress tumor vascularity.	Shih et al., 2012; Xia et al., 2012
miR-223	Down	stathmin1	Inhibit cell proliferation	Wong et al., 2008
miR-375	Down	YAP, AEG-1, ATG7	Inhibit tumorigenesis	Liu et al., 2010; He et al., 2012; Chang et al., 2012

the adenosine to inosine (A-to-I editing), which is mediated by the adenosine deaminase acting on dsRNA (ADAR) family of enzymes. The A-to-I editing can affect various types of RNA molecules including mRNAs, microRNAs, viral RNAs, etc. (Athanasiadis et al., 2004). RNA editing at the coding regions of the transcripts may lead to non-synonymous amino acid changes in the gene products, which might affect the biological functions of the proteins. Recent studies have linked A-to-I RNA editing to hepatocarcinogenesis. Through next-generation RNA sequencing technology, an A-to-I editing event within the AZIN1 transcript was identified in the tumor tissues from HCC patients. Hyper-editing of AZIN1 transcripts in the tumor cells resulted in a recording of AZIN1 protein from Serine to Glycine at codon 367. The edited AZIN1 showed strong oncogenic phenotypes on HCC cell lines and mouse models, compared with the wild type form. The frequency of RNA editing in the tumor tissues also significantly associated with the prognosis of HCC patients (Chen et al., 2013). The disrupted RNA editing was found to be mediated by differential expression of ADARs in HCC (Chan et al., 2013). Further characterization of the RNA editing events in HCC might help elucidate the pathogenesis of this disease (Li et al., 2013b).

EPIGENETIC ALTERATIONS IN HCC

Genetic alterations are irreversible changes that affect the DNA sequence of the genome. In contrast, epigenetic regulations do not change the sequence of the genome but affect the chromatin structure and gene transcription. Epigenetic regulations affect gene products at multiple

levels, including both transcriptional level and post-transcriptional regulation, which added great diversity to the gene regulation network. DNA methylation, histone modification, and recently emerging lncRNA, are major forms of epigenetic regulations. Alterations at cellular machineries governing those processes are frequently observed in cancer cells including HCC. The epigenetic alterations usually result in the activation of oncogenes or inactivation of tumor suppressor genes, which further contribute to malignant cancer hallmarks. Increasing evidences suggested that epigenetic alterations are evolving as an important mechanism in cancer initiation and progression (Mompalmer, 2003).

DNA methylation

In a normal cell, DNA methylation and demethylation is an important mechanism in regulating gene expression and chromatin structure. DNA methylase (DNMT) catalyze the methylation of cytosine at CpG islands at the promoter region of a gene. However, in tumor cells, the promoter methylation pattern is usually changed. Aberrant DNA methylation at the promoter region is an important mechanism of tumor suppressor gene inactivation. The hypermethylated CpG islands at the promoter region will prevent the binding of RNA polymerase and transcriptional factors, thus inhibit the transcription of the target genes. In addition, the hypermethylated protein will recruit m⁵CpG-binding domain (MBD) containing proteins, which will be an obstacle for the binding of transcriptional factors to the promoters, thus inhibit gene transcription (Hendrich and Bird, 1998).

In HCC, CpG island hypermethylation is frequently observed at the promoter region of important tumor suppressor genes. Suppressor of cytokine signaling (SOCS-1), which regulates the JAK/STAT signaling pathway, was found to be silenced in more than 60% of HCC patients due to promoter hypermethylation (Yoshikawa et al., 2001). The well-known tumor suppressor APC and E-cadherin were also hypermethylated in 53% and 49% of HCC patients, respectively (Yang et al., 2003). Methylation profiling of multi-step HCC tumors revealed that the number of genes methylated showed stepwise increase with the progression of cancer stage. The observation of tumor suppressor gene hypermethylation in the para-tumor liver tissues and cirrhotic livers indicated that aberrant promoter methylation occurs in the early stage of hepatocarcinogenesis and increased progressively during cancer progression (Lee et al., 2003). In addition, genome-wide DNA methylation analysis revealed that epigenetic silencing of multiple tumor suppressors in HCC could result in the activation of several oncogenic signaling pathways including Ras, JAK/STAT, and Wnt/ β -catenin (Calvisi et al., 2007).

There are several proposed hypotheses for the aberrant DNA methylation in cancer. One possible mechanism is the aberrant expression of DNMT1. As part of the DNA replication complex, DNMT1 transfer the methyl to the DNA immediately after DNA replication. In cancer cells, DNMT1 is usually abnormally expressed, and this will commit methylation errors during DNA replication (Vertino et al., 2002). Significant increase of DNMT1 was observed in HCC patients (Saito et al., 2003). In addition to DNMT1, which mainly accounts for the maintenance of methylation pattern of the genome, other DNMT family members such as DNMT3A and DNMT3B can directly add methyl groups to unmethylated DNA. DNMT3A and DNMT3B are responsible for novel methylation pattern formation in the genome (Okano et al., 1999). DNMT3A and DNMT3B were reported to be associated with hypermethylation of several important tumor suppressor genes, such as CDKN2A, CDKN2B, CDH1, and Rb1 (Mizuno et al., 2001). The expression of DNMT3A and DNMT3B are both significantly overexpressed in HCC compared with the non-cancerous liver tissues (Oh et al., 2007).

Histone modification and chromatin remodeling

Chromatin is the fundamental structure of the genome, which is constituted by nucleosome particles. The chromatin structure is important to gene transcription. In active transcription sites, the chromatin will be loosened, so that the DNA can be exposed to transcriptional factors for transcription initiation. This open chromatin structure is termed "euchromatin". In contrary, some of the chromatin structure is heavily condensed and the transcriptions of those genes within those regions are inhibited. The condensed chromatin structure is termed "heterochromatin". Thus, chromatin

structure is of critical importance in regulating gene expression in a temporal and spatial dependent manner (Wang et al., 2007a).

Histone modification is playing a central role in chromatin structure regulation. Covalent modification of histones with methylation or acetylation will result in the chromatin structural change and could be used as markers for chromatin structure. There are two histone modification markers which represent an active transcription. Trimethylation of H3 lysine 4 (H3K4Me3) is often observed at the promoter region of actively transcribed genes. Trimethylation of H3 lysine 36 (H3K36Me3) is also closely associated with active transcription. In contrary, trimethylation of H3 lysine 27 (H3K27Me3) and trimethylation of H3 lysine 9 (H3K9Me3) are associated with repressed transcription (Kouzarides, 2007). It is recognized that histone modification is catalyzed by several enzymes which modulate the histone markers. The histone modifiers include histone methyltransferases (HMT), histone acetyltransferase (HAT), and histone deacetylase (HDAC), etc. Abnormal expression of those histone modifiers which further drives epigenetic alterations are frequently observed in cancer cells. In HCC, overexpression of EZH2, which is the histone methyltransferase for H3K27Me3, has been proven to contribute to the malignant transformation and poor prognosis of HCC (Chen et al., 2007; Sudo et al., 2005). The P300/CBP-associated factor (PCAF), which is a well-known HAT, was expressed at low level in HCC, and has been proven to inhibit HCC tumorigenesis both *in vitro* and *in vivo* (Zheng et al., 2013). HDAC inhibitors have been suggested to specifically induce apoptosis in hepatoma cells but not in primary hepatocytes. And these results greatly supported the potential application of HDAC inhibitors in clinical treatment of HCC patients (Armeanu et al., 2005; Pathil et al., 2006).

In addition to histone modifiers, the ATP-dependent chromatin remodeling complex, which utilize ATP to mobilize nucleosomes along DNA, are also closely involved in tumorigenesis. The ATP-dependent chromatin remodeling family could be further divided into four subfamilies including: the SWI/SNF (Switching defective/, sucrose non-fermenting) family, the ISWI family (imitation SWI), the NuRD/CHD (Nucleosome remodeling and deacetylation/Chromodomain helicase, DNA binding) family, and the INO80 (inositol requiring 80) family (Wang et al., 2007). Whole-genome sequencing has identified recurrent somatic mutations in genes associated chromatin remodeling complex, including ARID1A, ARID2, and SMARCA4 (Guichard et al., 2012; Li et al., 2011). The frequently observed inactivating mutations indicated the important roles of chromatin remodeling complex in HCC development. The ATPase and putative DNA helicase RuvB-like 2 (RUVBL2) was found to be overexpressed in HCC and has contributed to the malignant transformation (Rousseau et al., 2007). Copy number loss or down-regulation of SWI/SNF chromatin remodelling subunit-BRG1 and BRM were also frequently observed in HCC

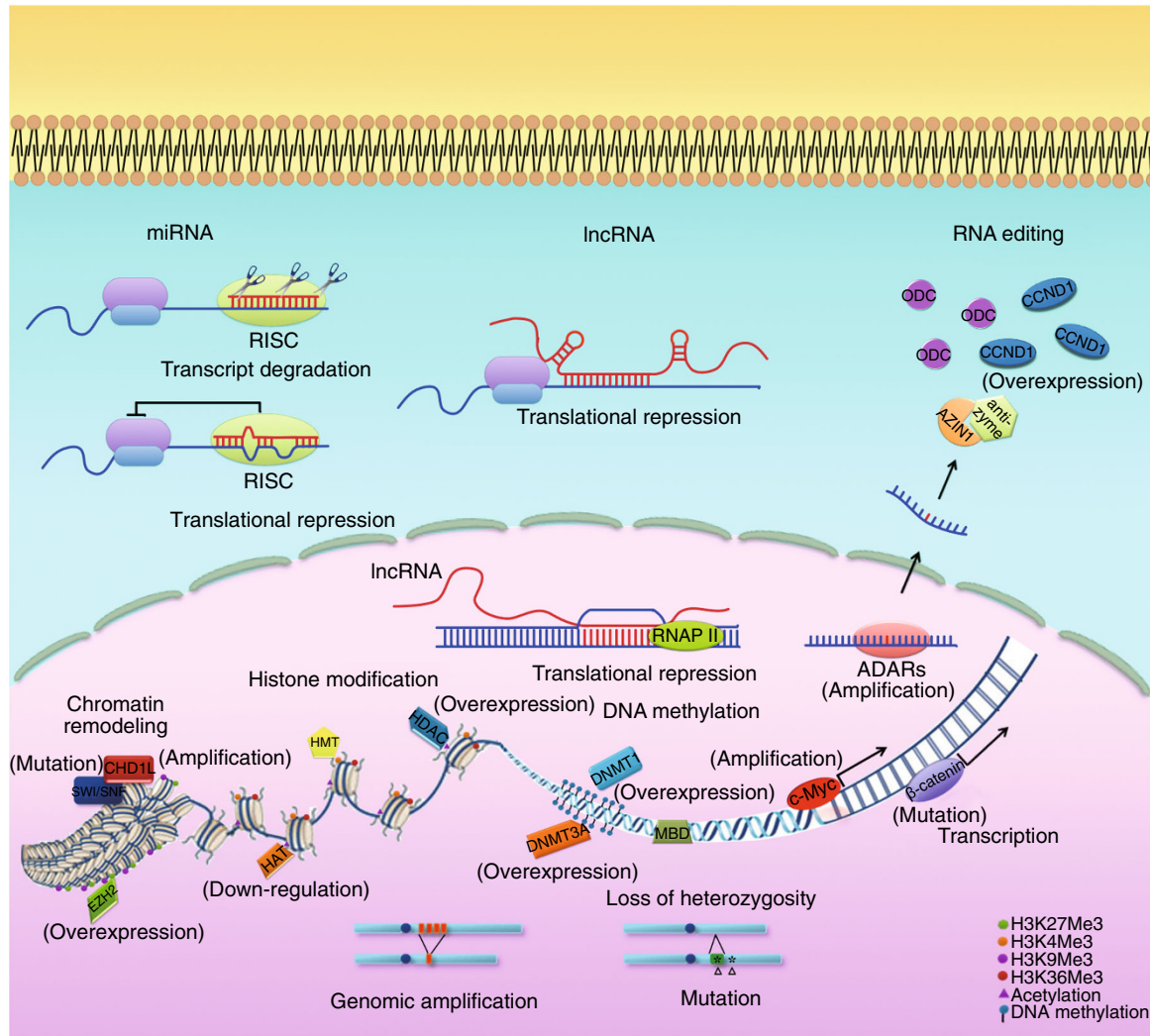


Figure 2. Genetic and epigenetic alterations in HCC. Genetic and epigenetic alterations interplay during cancer initiation and progression. The alterations exist at multiple levels including large chromosomal gain or loss, single nucleotide variations or mutations, overexpression or down-regulation of miRNAs and lncRNAs, disrupted RNA editing events, hyperactivation or inactivation of chromatin remodeling components, and aberrant DNA methylation at the promoter region of critical tumor suppressor genes et al.

patients (Endo et al., 2013). In addition, the CHD family member Chromodomain helicase DNA binding 1 like (CHD1L) has been proven to have diverse oncogenic roles in hepatocarcinogenesis (Chen et al., 2010).

Long non-coding RNAs (lncRNAs)

A large number of non-protein coding transcripts exist in the genome. In the past, those long non-coding RNAs were considered as “rubbish” of the genome for their unknown functions. Recently, emerging evidences suggested that lncRNAs might play important roles in regulating gene expression at post-transcriptional level. lncRNAs can regulate gene transcription either through directly binding to the RNA polymerase II, or modifying the activity of the

transcriptional co-regulators (Mercer et al., 2009). In addition to transcriptional regulation, lncRNAs can also control the post-transcriptional mRNA processing such as mRNA splicing and translation. Furthermore, lncRNAs were also reported to be involved in regulating histone methylation and chromatin remodeling, which are the most important epigenetic regulatory machinery in regulating gene expression (Guttman and Rinn 2012). Altered expression of lncRNAs has been observed in tumors including HCC and they are suggested to play critical roles during tumorigenesis. High expression of lncRNA-HEIH is significantly associated with HCC recurrence and poor prognosis. *In vitro* and *in vivo* functional studies revealed that the overexpression of lncRNA-HEIH can promote HCC tumorigenesis and might function through EZH2 (Yang et al., 2011a). In addition,

overexpression of Long Non-coding RNA HOTAIR and MALAT-1 could help predict tumor recurrence and prognosis of HCC patients (Lai et al., 2012; Yang et al., 2011b). All these evidences indicated that lncRNAs might be important in HCC initiation and progression.

SUMMARY AND PERSPECTIVES

Like other solid tumors, HCC is characterized with multiple hallmarks including sustained proliferation, evading growth suppressive signals, metastasis to other organs, promoting angiogenesis, tumor-promoting microenvironment, cancer stem cell properties, and energy metabolism switch, etc. Genetic and epigenetic alterations interplay during cancer initiation and progression. The genomic changes vary from large chromosomal gain or loss to single nucleotide variations or mutations. Genetic alterations are irreversible alterations, which could be observed as early as in the pre-neoplastic stages. The early onset of genetic alterations indicated that they might be the tumor initiating steps in the development of cancer. Chromosome instability is the most common type of genetic alteration. Chromosome 1q and 8q are frequently amplified, while chromosome 1p, 4q, 6q, 9p, 16p, 16q, and 17p are frequently lost in HCC. Those hot regions usually harbor important oncogenes or tumor suppressor genes, which might significantly contribute to hepatocarcinogenesis. In addition to large chromosomal segmental changes, single nucleotide changes in the genome also make a substantial contribution to cancer progression. Nucleotide changes known as mutations or variations can lead to either gain-of-function or loss-of-function of oncogenes and tumor suppressor genes. Non-coding nucleotide changes can also affect gene transcription, and post-transcriptional regulations of critical tumor related genes, which may directly trigger oncogenesis or enhance cancer risk. Epigenetic alteration is another important mechanism for oncogenesis. Epigenetic regulation includes a wide range of regulations at transcriptional or post-transcriptional levels, such as DNA methylation, histone modification, chromatin remodeling, and lncRNAs. Alterations at the epigenetic regulation machinery may lead to disrupted gene expression, which can also cause the activation of oncogenes or inactivation of tumor suppressor genes. The genetic and epigenetic alterations in HCC are summarized in Fig. 2. Small molecules or monoclonal antibodies, which specifically target the altered onco-proteins, have already been proven to be efficient in treating several types of cancer. For example, imatinib, which specifically target the BCR-ABL fusion kinase, is used in treating chronic myeloid leukemia; trastuzumab, a monoclonal antibody targeting the amplified tyrosine kinase receptor HER2, is used to treat advanced-stage breast cancer. However, the targeted therapies which are effective in treating HCC are still limited. Better understanding and characterization of novel genetic and epigenetic alterations, which are important

to hepatocarcinogenesis, may help understand the molecular pathogenesis of HCC, as well as providing novel therapeutic targets for HCC treatment.

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ABBREVIATIONS

Ang2, angiopoietin-2; bFGF, basic fibroblast growth factor; CGH, comparative genomic hybridization; CSC, cancer stem cell; ECM, extracellular matrix; GWAS, genome-wide association study; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; lncRNAs, long non-coding RNAs; LOH, loss of heterozygosity; MMP, matrix metalloproteinases; PDGF, platelet-derived growth factor; RIZ1, Rb-interacting zinc finger 1; TAM, tumor associated macrophage; TCA, tricarboxylic acid.

COMPLIANCE WITH ETHICS GUIDELINES

Ming Liu, Lingxi Jiang, and Xin-Yuan Guan declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by the any of the authors.

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